Bull Environ Contam Toxicol
DOI 10.1007/s00128-010-9951-x

Toxicity Evaluation of a Conservation Effects Assessment Program Watershed, Beasley Lake, in the Mississippi Delta, USA

Richard E. Lizotte Jr. · Scott S. Knight · Charles M. Cooper

Received: 21 May 2009 / Accepted: 26 February 2010

Abstract Beasley Lake was assessed monthly in 2005 for biological impairment from 17 historic and current-use pesticides in water and leaf litter using *Hyalella azteca* (Saussure). Sixteen pesticides were detected in both water and leaf litter with peak detections in spring and summer. Detections ranged from 1–125 ng L^{-1} in water and 1–539 ng g^{-1} OC in leaf litter. Ten-day *H. azteca* survival and growth (mg dw) bioassay results indicated no adverse effects on survival or growth in *H. azteca* exposed to water or leaf litter. Rather, enhanced growth occurred in both lake water and leaf litter exposures for 8 and 6 months, respectively.

Keywords CEAP · Pesticides · Lake water · Leaf litter · *Hyalella azteca*

Beasley Lake, located in Sunflower County in the Mississippi Delta (latitude 33°24′15 N, longitude 90°40′05 W), was chosen as a Conservation Effects Assessment Project (CEAP) watershed in part because of the large data base of background information and its contributions to the understanding of best management practices (BMP) effectiveness on watershed scales (Nett et al. 2004; Zablotowicz et al. 2006). The watershed has a total drainage area of approximately 915 ha and a lake surface area of about 25-30 ha. From 1995 to 2001 the watershed surrounding the lake was primarily farmed in conventional tillage cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybeans (*Glycine max* [L.] Merr.). These practices were followed with the implementation of reduced tillage in 2001 and enrollment into the Conservation Reserve Program (CRP) in 2003 consisting of 113 ha planted with cottonwood (*Populus deltoides* Bartr. ex. Marsh.) trees (Locke et al. 2008).

Historically, Mississippi Delta lakes, valued for their productivity and recreational use have not escaped impairment due to pesticide contamination (Cooper et al. 2003). As a result, water quality and ecological diversity have declined and their popularity has decreased. Recent attempts at rehabilitating Beasley Lake to restore productivity and recreational value has shown some success (Locke et al. 2008). However, there is a need for assessing the degree of remaining pesticide contamination and possible biological impairment. The purpose of this study was to ascertain the degree of remaining pesticide contamination and biological impairment in Beasley Lake using chemical analyses and 10-day *Hyalella azteca* bioassays.

Materials and Methods

Aqueous and leaf litter samples were collected monthly during 2005 at approximately the mid-point in Beasley Lake. Six liters of water and 20 g simulated leaf litter (conditioned In Situ two weeks prior to sampling) using post-abscission Florida maple leaves (*Acer barbatum* Michx.) in polyester mesh bags (Moore et al. 2007a) were sampled on site, preserved on ice and transported to the USDA-ARS National Sedimentation Laboratory, Oxford, MS, for selected lake water quality analysis, pesticide analysis and toxicity bioassays. Because of the very soft nature of the lake water, samples were hardness adjusted to between 80 and 100 mg L^{-1} as $CaCO_3$ with sodium bicarbonate and calcium chloride. Upon arrival, a single

R. E. Lizotte Jr. (✉) · S. S. Knight · C. M. Cooper
USDA-ARS National Sedimentation Laboratory,
PO Box 1157, Oxford, MS 38655, USA
e-mail: richard.lizotte@ars.usda.gov

Published online: 14 March 2010

1 L lake water sample for water quality analysis was processed and analyzed for total NH_4-N, total NO_3-N, total NO_2-N, total suspended solids (TSS), chlorophyll *a*, and dissolved organic carbon (DOC) concentrations according to APHA (2005). In addition, one 4 L aqueous sample for pesticide analysis was immediately extracted using ethyl acetate and KCl according to Bennett et al. (2000) and Cooper et al. (2003). Leaf litter samples were dried for 48 h. Aqueous and leaf litter samples were analyzed for 17 current and historic-use pesticides and selected associated metabolites. Analytical chemistry was conducted according to Bennett et al. (2000) using a Hewlett-Packard 6890 gas chromatograph equipped with dual HP 7683 ALS autoinjectors. Leaf litter samples were ground, prewetted with ultrapure water followed by the addition of ethyl acetate. The mixture was sonicated and centrifuged (2,000–2,500 rpm). The extract was concentrated to near dryness (1 mL) using a nitrogen evaporator and solvent exchanged into hexane. Levels of detection for aqueous and leaf litter analyses were 1 ng L^{-1} and 1 ng g^{-1} OC, respectively. Extraction efficiencies of all fortified samples analyzed using quality assurance/quality control protocols were ≥90%.

Monthly ten-day static, non-renewal, aqueous toxicity tests using *H. azteca* were conducted, with modifications, according to methods described by Deaver and Rodgers (1996) and USEPA (2000) protocols. Individuals 4–5 days old were used for all tests. Each aqueous exposure consisted of 200 mL from a lake sample placed in four replicate exposure chambers (250 mL borosilicate glass beakers) per site. Ten *H. azteca* were placed in each exposure chamber along with five, 6 mm diameter Florida maple leaf discs as substrate and food. Additional feeding of 1 mL of a 1:1 mixed suspension of rabbit chow:Tetramin® flake food (10 mg L^{-1}) occurred at test initiation and every two days. Leaf-litter exposures consisted of 200 mL of overlying water, free from priority pollutants, obtained from The University of Mississippi Field Station (UMFS) (Deaver and Rodgers 1996) and five, 6 mm diameter Florida maple leaf discs as a substrate and food. Because of the very soft nature of UMFS water, samples were hardness adjusted to between 80 and 100 mg L^{-1} as $CaCO_3$ with sodium bicarbonate and calcium chloride. Control leaf-litter was preconditioned (two weeks) in mesh bags at the UMFS. Toxicity tests were conducted in a Powers Scientific, Inc. Animal Growth Chamber with a 16:8 (light:dark) h photoperiod and a set temperature of 23 ± 1°C. Measured physical and chemical water characteristics for aqueous and leaf litter bioassays were temperature, pH, dissolved oxygen, conductivity, hardness, and alkalinity (APHA 2005). Bioassay endpoints measured were survival and growth (mg dw). Bioassay data were analyzed using descriptive statistics and *t*-tests versus controls for each month. Statistical significance level was set at 5 percent ($p \leq 0.05$) for all analyses (Steel et al. 1997). Data analysis was conducted using SigmaStat® v.2.03 statistical software (SPSS 1997).

Results and Discussion

Monthly Beasley Lake selected physical and chemical water quality data are presented in Table 1. Peak nitrogen, TSS and turbidity occurred during winter and spring months coinciding with peak monthly rainfall and concomitant runoff then decreasing during the drier summer and fall months (Nett et al. 2004). Chlorophyll *a* (indirect measure of primary productivity) fluctuated in conjunction with TSS and turbidity and DOC fluctuated throughout the year but was stable during the fall months in association with allochthonous input (post-abscission leaf litter).

Pesticide analysis revealed seasonal variation in lake water and leaf litter contamination (Table 2). Of the 17 pesticides and metabolites assessed, only alachlor and pendimethalin were not detected in lake water and leaf litter, respectively. Seasonally, peak current-use pesticide contamination in lake water and leaf litter occurred during spring and summer coinciding with agricultural pest-management practices. In contrast, peak contamination from legacy pesticides (dieldrin, DDT and metabolites) in lake water occurred from late spring through fall and in leaf litter from late winter through spring. Greatest herbicide concentrations occurred with atrazine in both lake water and leaf litter. Greatest current-use insecticide concentrations occurred with chlorpyrifos in lake water and pyrethroids bifenthrin and λ-cyhalothrin in leaf litter. Of the legacy pesticides, DDT occurred in the greatest concentrations in both lake water and leaf litter. Previously reported pesticide contamination in Beasley Lake water from 1999 to 2000 (Table 3; Cooper et al. 2003) showed much greater concentrations of current-use herbicides (e.g., atrazine, cyanazine and metolachlor) and insecticides (e.g., methyl parathion, bifenthrin and λ-cyhalothrin).

Ten-day toxicity bioassay water quality data for all tests were within parameters for acute aqueous bioassays according to USEPA (2000). Parameters for lake water and leaf litter bioassays were as follows: temperature (°C), 23.5 ± 0.3; dissolved oxygen (mg L^{-1}), 7.6 ± 0.5; pH, 8.1 ± 0.3; alkalinity (mg L^{-1} as $CaCO_3$), 54.2 ± 17.6; hardness (mg L^{-1} as $CaCO_3$), 95.0 ± 16.1; conductivity (µS cm^{-1}), 327.5 ± 57.3. Parameters for bioassay UMFS control water were as follows: temperature (°C), 23.6 ± 0.3; dissolved oxygen (mg L^{-1}), 7.6 ± 0.4; pH, 8.2 ± 0.2; alkalinity (mg L^{-1} as $CaCO_3$), 50.6 ± 12.7; hardness (mg L^{-1} as $CaCO_3$), 99.0 ± 17.6; conductivity (µS cm^{-1}), 343.7 ± 44.0; NH_4 (µg L^{-1}), 27 ± 40; NO_3 (µg L^{-1}), 339 ± 390; NO_2 (µg L^{-1}), 9 ± 6; TSS (mg L^{-1}), 10 ± 11;

Table 1 Beasley Lake selected water quality characteristics over a 12-month period from a single monthly sample

Month	NH_4 μg L^{-1}	NO_3 μg L^{-1}	NO_2 μg L^{-1}	TSS[a] mg L^{-1}	Turbidity NTU	Chlorophyll *a* μg L^{-1}	DOC[b] mg L^{-1}
J	0	254	32	200	210	26.3	1.8
F	0	327	25	202	153	4.5	11.2
M	0	86	10	142	137	5.7	1.9
A	0	225	11	144	127	3.5	3.1
M	0	124	20	34	48.6	41.4	1.9
J	0	235	0	28	7.9	29	3.2
J	0	21	10	16	4.9	12.6	10.3
A	0	38	0	0	2.8	35.6	4.9
S	0	30	0	17	4.1	19.7	7.7
O	0	30	19	9	6.7	35.4	7.9
N	5	88	0	11	12.6	27.4	7.5
D	0	169	0	9	14.4	54.2	7.2

[a] *TSS* total suspended solids

[b] *DOC* dissolved organic carbon

Table 2 Lake water (ng L^{-1}) and leaf litter (ng g^{-1} OC) pesticide concentrations in Beasley Lake over a 12-month period (–, below detection limit of 1 ng L^{-1} lake water and 1 ng g^{-1} OC leaf litter)

Pesticide	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
Trifluralin	–[a], –[b]	–, 2	–, 4	–, 3	–, 7	–, –	–, –	6, 24	–, –	–, 7	–, –	–, 7
Pendimethalin	–, –	–, –	–, –	–, –	2, –	–, –	–, –	–, –	–, –	–, –	–, –	–, –
Atrazine	–, 539	–, –	–, 298	–, 40	68, 36	36, 18	–, 561	125, 395	–, 378	25, –	–, 311	22, –
Cyanazine	–, 1	–, –	–, –	–, 1	2, –	–, 1	–, –	–, –	–, –	–, –	–, –	–, –
Alachlor	–, 10	–, 1	–, 7	–, 7	–, 16	–, 27	–, 14	–, 38	–, 9	–, 8	–, 5	–, –
Metolachlor	–, –	–, –	–, –	–, 25	27, –	10, 2	–, 2	–, –	–, –	–, –	–, –	–, –
Methyl parathion	–, 1	–, –	9, 12	–, –	–, –	15, –	–, –	–, 11	–, 12	–, –	–, 11	–, –
Chlorpyrifos	–, 23	–, –	–, –	–, –	38, –	39, –	–, –	–, –	–, –	–, –	21, –	26, –
Chlorfenapyr	–, –	–, –	–, 1	–, –	3, –	3, –	3, 2	2, –	2, –	–, –	–, –	2, –
Bifenthrin	–, –	–, –	–, –	–, 25	–, 25	–, –	4, 27	–, 32	13, 19	–, 19	–, –	–, –
λ-cyhalothrin	–, 33	–, –	–, –	–, –	–, –	3, –	15, –	–, –	13, –	10, –	–, –	–, –
Fipronil	–, 3	–, –	–, 2	–, –	–, –	4, –	–, –	9, –	–, 3	3, –	–, –	–, –
Fipronil sulfone	–, –	–, –	–, –	–, 7	4, –	4, –	3, –	4, 1	4, –	2, –	–, –	2, –
Dieldrin	–, 3	–, –	1, 1	–, 3	2, –	1, 9	2, –	1, 6	–, 15	1, 13	–, –	3, –
p,p′-DDT	10, 2	–, 2	7, 3	–, 32	42, –	25, –	15, 27	37, –	26, 4	25, –	23, 11	42, –
p,p′-DDD	–, –	–, –	–, 1	–, 3	6, 2	5, –	6, –	4, –	5, –	2, –	–, –	–, –
p,p′-DDE	–, 3	–, –	–, 1	–, 3	3, –	2, 3	4, 3	1, –	2, 5	2, 2	2, –	3, –

[a] Lake water pesticide concentration

[b] Leaf litter pesticide concentration

turbidity (NTU), 8.5 ± 8.3; chlorophyll *a* (μg L^{-1}), 4.6 ± 6.7; DOC (mg L^{-1}), 1.0 ± 0.2. *H. azteca* ten-day survival was not significantly ($p > 0.05$) affected by exposure to either lake water or leaf litter during any month examined (Table 4). Lake water survival responses of this study were similar to those observed by Moore et al. (2007b) in Beasley Lake water exposures conducted in March 2001 during which pesticide contamination was much greater (nine pesticides detected ranging from 2 to 105 ng L^{-1}). Few studies exist concerning effects of pesticide-contaminated leaf litter on *H. azteca* for the 17 pesticides examined in this study (Moore et al. 2007a; Maul et al. 2008). In this study, measured pesticide concentrations in leaf litter were not sufficient to cause significant lethality after 10 days.

Table 3 Mean ± SD annual lake water (ng L^{-1}) pesticide concentrations in Beasley Lake during 1999, 2000, and 2005 (detection limit of 1 ng L^{-1}; NM, not measured)

Pesticide	1999[a]	2000[a]	2005[b]
Trifluralin	12 ± 19	11 ± 5	1 ± 2
Pendimethalin	11 ± 31	6 ± 4	0 ± 1
Atrazine	597 ± 1,031	414 ± 432	23 ± 39
Cyanazine	41 ± 38	227 ± 217	0 ± 1
Alachlor	0 ± 1	16 ± 29	0 ± 0
Metolachlor	1,355 ± 2,876	112 ± 82	3 ± 8
Methyl parathion	5 ± 9	32 ± 24	2 ± 5
Chlorpyrifos	2 ± 4	6 ± 9	10 ± 16
Chlorfenapyr	5 ± 9	6 ± 4	1 ± 1
Bifenthrin	10 ± 17	21 ± 21	1 ± 4
λ-cyhalothrin	14 ± 16	12 ± 13	3 ± 6
Fipronil	4 ± 0	4 ± 3	1 ± 3
Fipronil sulfone	4 ± 0	3 ± 2	2 ± 2
Dieldrin	3 ± 6	4 ± 2	1 ± 1
p,p′-DDT	NM	NM	21 ± 15
p,p′-DDD	4 ± 6	17 ± 9	2 ± 3
p,p′-DDE	8 ± 20	5 ± 3	2 ± 1

[a] From Cooper et al. (2003)

[b] Present study

Table 4 Survival (%) of *Hyalella azteca* exposed for 10 days to Beasley Lake water and leaf litter over a 12-month period

Month	Water		Leaf litter	
	Control	Beasley	Control	Beasley
J	100 ± 0	100 ± 0	95 ± 6	85 ± 6
F	90 ± 12	93 ± 15	98 ± 5	100 ± 0
M	95 ± 6	80 ± 8	98 ± 5	95 ± 10
A	93 ± 10	85 ± 6	88 ± 5	90 ± 8
M	93 ± 10	95 ± 10	95 ± 6	88 ± 10
J	93 ± 15	90 ± 12	95 ± 6	93 ± 5
J	98 ± 5	90 ± 12	100 ± 0	100 ± 0
A	93 ± 10	93 ± 15	98 ± 5	90 ± 0
S	90 ± 8	90 ± 14	100 ± 0	98 ± 5
O	100 ± 0	100 ± 0	95 ± 6	100 ± 0
N	100 ± 0	93 ± 10	100 ± 0	98 ± 5
D	98 ± 5	100 ± 0	98 ± 5	98 ± 5

Sub-lethal ten-day animal growth impairment was not observed from exposure to either lake water or leaf litter during any month examined (Table 5). Rather, significant ($p < 0.05$) enhanced growth was observed in both lake water and leaf litter exposures during at least six months (March through July and November). In contrast, Moore et al. (2007b) observed growth impairment in *H. azteca* exposed to Beasley Lake water in March 2001 in association with measured concentrations of trifluralin, atrazine, and methyl parathion. Because Moore et al. (2007b) used the same methods for controls and Beasley Lake exposures in 2001 as the present study, the two studies are directly comparable. Moore et al. (2007b) observed a 21% decrease versus controls in growth as length (3.21 vs. 2.54 mm) and a 33% decrease in growth as dry weight (0.03 vs. 0.02 mg) in animals exposed to Beasley Lake water. By comparison, the present study observed a 125% increase versus controls in growth as dry weight (0.04 vs. 0.09 mg) and a 350% increase in growth versus March 2001 as dry weight (0.02 vs. 0.09 mg) in *H. azteca* exposed to Beasley Lake water in March 2005 (Moore et al. 2007b). Despite the importance of leaf litter as a food source for the epibenthic detritivore, *H. azteca*, (De March 1981) few studies have examined the effect of contaminated detritus on growth (Sundberg et al. 2006). Due to the paucity of data, comparisons are limited. However, within the frame of the current study, measured pesticide contamination in leaf litter did not elicit growth impairment during any month.

Table 5 Growth (mg dw) of *Hyalella azteca* exposed for 10 days to Beasley Lake water and leaf litter over a 12-month period

Month	Water		Leaf litter	
	Control	Beasley	Control	Beasley
J	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02
F	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.01
M	0.04 ± 0.01	0.09 ± 0.02*	0.04 ± 0.02	0.07 ± 0.01*
A	0.02 ± 0.01	0.11 ± 0.03*	0.03 ± 0.02	0.12 ± 0.03*
M	0.02 ± 0.01	0.09 ± 0.02*	0.03 ± 0.01	0.08 ± 0.01*
J	0.03 ± 0.01	0.10 ± 0.04*	0.03 ± 0.01	0.10 ± 0.01*
J	0.03 ± 0.01	0.06 ± 0.01*	0.02 ± 0.01	0.05 ± 0.01*
A	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
S	0.02 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.01
O	0.03 ± 0.01	0.07 ± 0.01*	0.03 ± 0.02	0.05 ± 0.01
N	0.04 ± 0.01	0.06 ± 0.01*	0.04 ± 0.01	0.08 ± 0.01*
D	0.04 ± 0.01	0.07 ± 0.01*	0.04 ± 0.01	0.05 ± 0.01

* Statistically significantly different versus control ($p < 0.05$)

Results of this study show an improvement in Beasley Lake water quality and concomitant responses of *H. azteca* after 10-day exposures in comparison with observed conditions prior to implementation of conservation practices. A combination of both agricultural best management practices and conservation practices has resulted in significant improvement in lake water quality for the sentinel species, *Hyalella azteca*.

Acknowledgments Appreciation is extended to S. Smith, C. Bryant, V. Campbell, D. McChesney, L. Brooks, A. Delmastro, and L. Arbuckle for analytical assistance as well as C. Bryant and M. Haasch for reviewing an earlier version of the manuscript. Mention of

equipment, software or a pesticide does not constitute an endorsement for use by the US Department of Agriculture nor does it imply pesticide registration under FIFRA as amended. All programs and services of the USDA are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, marital status, or handicap.

References

American Public Health Association (APHA) (2005) Standard methods for the examination of water and wastewater, 21st edn. Washington, DC

Bennett ER, Moore MT, Cooper CM, Smith S (2000) Method for the simultaneous extraction and analysis of two current use pesticides, atrazine and lambda-cyhalothrin, in sediment and aquatic plants. Bull Environ Contam Toxicol 64:825–833

Cooper C, Smith S, Moore M (2003) Surface water, ground water and sediment quality in three oxbow lake watersheds in the Mississippi delta agricultural region: pesticides. Int J Ecol Environ Sci 29:171–184

De March BGE (1981) Hyalella azteca (Saussure). In: Lawrence SG (ed) Manual for the culture of freshwater invertebrates. Canadian Spec Publ Fish Aquat Sci 54:61–77

Deaver E, Rodgers JH (1996) Measuring bioavailable copper using anodic stripping voltammetry. Environ Toxicol Chem 15:1925–1930

Locke MA, Knight SS, Smith S, Cullum RF, Zablotowicz RM, Yuan Y, Bingner RL (2008) Environmental quality research in the Beasley Lake watershed, 1995–2007: succession from conventional to conservation practices. J Soil Water Conserv 63:430–442. doi:10.2489/jswc.63.6.430

Maul JD, Trimble AJ, Lydy MJ (2008) Partitioning and matrix-specific toxicity of bifenthrin among sediments and leaf-sourced organic matter. Environ Toxicol Chem 27:945–952

Moore MT, Lizotte RE, Smith S (2007a) Reponses of *Hyalella azteca* to a pyrethroid mixture in a constructed wetland. Bull Environ Contam Toxicol 78:245–248. doi:10.1007/s00128-007-9135-5

Moore MT, Lizotte RE, Knight SS, Smith S, Cooper CM (2007b) Assessment of pesticide contamination in three Mississippi delta oxbow lakes using *Hyalella azteca*. Chemosphere 67:2184–2191. doi:10.1016/j.chemosphere.2006.12.026

Nett MT, Locke MA, Pennington DA (2004) Water quality assessments in the mississippi delta: regional solutions, national scope. ASC symposium series 877, Washington, DC

Statistical Package for the Social Sciences (SPSS), Inc. (1997) SigmaStat for Windows version 2.03

Steel RGD, Torrie JH, Dickey DA (1997) Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill, Inc., New York p 665

Sundberg SE, Hassan SM, Rodgers JH (2006) Enrichment of elements in detritus from a constructed wetland and consequent toxicity to *Hyalella azteca*. Ecotox Environ Saf 64:264–272. doi:10.1016/j.ecoenv.2006.02.010

US Environmental Protection Agency (USEPA) (2000) Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. USEPA 600/R-99/064

Zablotowicz RM, Locke MA, Krutz LJ, Lerch RN, Lizotte RE, Knight SS, Gordon RE, Steinriede RW (2006) Influence of watershed system management on herbicide concentrations in Mississippi Delta oxbow lakes. Sci Total Environ 370:552–560. doi:10.1016/j.scitotenv.2006.08.023